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(54) Title: HUMAN HEPARANASE-RELATED POLYPEPTIDE AND NUCLEIC ACID

(57) Abstract: The present invention relates to newly identified polynucleotides, and polypeptides encoded by such polynucleotides, the use of such polypeptides, as well as the production of such polynucleotides and polypeptides. More particularly, a polypeptide of the present invention is a heparanase-related endoglucuronidase. The invention also relates to vectors and host cells comprising a polynucleotide of the invention. Furthermore, the invention relates to antibodies directed to polypeptides according to the present invention and to pharmaceutical compositions and diagnostic reagents comprising such antibodies, polypeptides or polynucleotides. The invention further relates to a method of altering, modifying or otherwise modulating the level of expression of the heparanase-related endoglucuronidase in a cell or in a organism. A further aspect of the invention are assay systems suitable for identifying modulators, e.g. agonists or antagonists of such polypeptides.

Human heparanase-related polypeptide and nucleic acid

Description

FIELD OF THE INVENTION

The present invention relates to newly identified polynucleotides, and polypeptides encoded by such polynucleotides, the use of such polypeptides, as well as the production of such polynucleotides and polypeptides. More particularly, a polypeptide of the present invention is a heparanase-related endoglucuronidase. The invention also relates to vectors and host cells comprising a polynucleotide of the invention. Furthermore, the invention relates to antibodies directed to polypeptides according to the present invention and to pharmaceutical compositions and diagnostic reagents comprising such antibodies, polypeptides or polynucleotides. The invention further relates to a method of altering, modifying or otherwise modulating the level of expression of the heparanase-related endoglucuronidase in a cell or in a organism. A further aspect of the invention are assay systems suitable for identifiying modulators, e.g. agonists or antagonists of such polypeptides.

BACKGROUND OF THE INVENTION

Extracellular matrix (ECM) and basement membrane (BM) proteins are embedded in a fibre meshwork consisting mainly of heparan sulfate proteoglycan (HSPG). HSPG´s are prominent compounds of blood vessels (subendothelial basement membrane) which support the endothelial cells and stabilize the structure of the capillary wall. Expression of heparanase, an endo-ß-D-glucuronidase, in platelets, placental trophoblasts, and leucocytes demonstrates the normal function of heparanase in embryonic

morphogenesis, wound healing, tissue repair, and inflammation. In concert with ECM-digesting proteases heparanase enables cells to traverse the basement membrane and releases heparin-binding growth factors (e.g. bFGF, VEGF) which are stored in the ECM (Finkel et al., Science 285 (1999), 33-34; Eccles, Nature Med. 5 (1999), 735-736).

Heparanase, which has recently been cloned by 4 independent groups (Vlodavsky et al., Nature Med. 5 (1999), 793-802; Hulett et al., Nature Med. 5 (1999), 803-809; Toyoshima and Nakajima, J. Biol. Chem. 274 (1999), 24153-24160; Kussie et al., Biochem. Biophys. Res. Comm. 261 (1999), 183-187), is expressed as a 65 kDa precursor protein which becomes N-terminally processed into the 50 kDa active enzyme. Recombinant expression of the active enzyme has been demonstrated in CHO, NIH 3T3 and in COS-7 cells. Although several apparently different heparanase activities have been described previously, the 4 groups which cloned the heparanase cDNA from different sources (normal and tumor cells) reported on identical cDNA sequences.

Several lines of evidence demonstrate an involvement of ECM degrading glucuronidases in tumor growth and metastasis formation: (1) Heparanase was shown to be preferentialy expressed on the mRNA and the protein level in human tumor tissues as compared to the corresponding normal tissue, e.g. invasive ductal carcinoma of the breast, hepatocellular carcinoma, ovary adenocarcinoma; squamous carcinoma of the cervix, colon adenocarcinoma (Vlodavsky et al., supra). (2) Increased levels of heparanase were shown in sera and urine of metastatic tumor-bearing animals and in cancer patients (Vlodavsky et al., supra). (3) Heparanase mRNA expression and enzyme acitivity correlates with metastatic potential of human and rat breast tumor cell lines (Vlodavsky et al., supra; Hulett et al., supra). (4) Low metastatic tumor cells aquire a highly metastatic phenotype upon transfection of heparanase cDNA, e.g. shown for murine T lymphoma L5178Y and mouse B16-F1 melanoma (Vlodavsky et al.,

supra). (5) The sulfated oligosaccharide PI-88 (phosphomannopentaose SO_4), which inhibits heparanase activity, inhibits primary tumor growth, metastasis formation, and tumor vascularization (Parish et al., Cancer Res. 59 (1999), 3433-3441).

SUMMARY OF THE INVENTION

The present invention provides a new isolated nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding a polypeptide having endoglucuronidase enzymatic activity or a fragment thereof.

The present invention further relates to a polypeptide encoded by the polynucleotide, a functional fragment or a functional derivative or a functional analog thereof.

Another aspect of the invention relates to a process for preparing such a polypeptide or such a polynucleotide.

A further aspect of the invention relates to a recombinant vector comprising such a polynucleotide, preferably in operative linkage to an expression control sequence and a host cell transformed with such a recombinant vector.

Moreover, the present invention relates to a method of altering, modifying or otherwise modulating the level of expression of such a polypeptide or such a polynucleotide in a cell or in a organism.

Another aspect of the present invention relates to a method of diagnosis utilizing such a polynucleotide, or fragment or derivative thereof, or polypeptide, or fragment or derivative thereof.

Furthermore, the present invention relates to antibodies specifically recognizing and binding to such a polypeptide and to a method of diagnosis utilizing such an antibody.

Moreover, the present invention relates to pharmaceutical compositions comprising such a polynucleotide or such a polypeptide or such an antibody or a fragment thereof, and to a method of treatment comprising administration of such a polynucleotide or polypeptide or antibody or a fragment thereof.

A yet further aspect of the present invention relates to a method for identifying a substance capable of modulating the biological activity of such a polypeptide, and substances obtainable by such a method.

DETAILED DESCRIPTION OF THE INVENTION

An isolated nucleic acid molecule comprising a nucleotide sequence encoding or complementary to a sequence encoding a polypeptide having the enzymatic activity of an endoglucuronidase is provided.

In a preferred embodiment thereof an isolated nucleic acid molecule according to the present invention is the nucleic acid molecule comprising (a) at least the protein coding portion of the nucleotide sequence set forth in SEQ ID NO 1, (b) a nucleotide sequence corresponding to the sequence of (a) in the scope of the degeneracy of the genetic code or (c) a nucleotide sequence hybridizing under stringent conditions to the nucleotide sequence of (a) and/or (b).

The present invention further provides a polypeptide encoded by the nucleic acid molecule according to the present invention. Preferably, the polypeptide comprises (a) the amino acid sequence set forth in SEQ ID NO 2 or (b) an amino acid sequence having an identity of at least 70%,

preferably at least 85% and more preferably at least 95% to the amino acid sequence of (a).

In addition to the nucleotide sequence as set forth in SEQ ID NO 1 and a nucleic acid sequence corresponding thereto in the scope of the degeneracy of the genetic code, the present invention encompasses also a nucleotide sequence which hybridizes under stringent conditions with one of the sequences as defined above. The term "hybridization under stringent conditions" according to the present invention is defined according to Sambrook et al., Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory Press (1989), 1.101-1.104. Preferably, hybridization under stringent conditions means that after washing for 1 h with 1 x SSC and 0.1% SDS at 50°C, preferably at 55°C, more preferably at 62°C and most preferably at 68°C, particularly for 1 h in 0.2 x SSC and 0.1% SDS at 50°C, preferably at 55°C, more preferably at 62°C and most preferably at 68°C a positive hybridization signal is observed. A nucleotide sequence which hybridizes under the above washing conditions with the nucleotide sequence as set forth in SEQ ID NO 1 or a nucleotide sequence corresponding thereto in the scope of the degeneracy of the genetic code is encompassed by the present invention.

Preferably, the nucleotide sequence according to the invention is a DNA, e.g. a cDNA, genomic DNA or synthetic DNA, which may be double-stranded or single-stranded, and if single-stranded may be the coding or non-coding (anti-sense) strand. It can, however, comprise an RNA, e.g. an mRNA, pre-mRNA and synthetic RNA either the coding or the non-coding (anti-sense) strand or a nucleic acid analog such as a peptidic nucleic acid. Particularly preferred, the nucleotide sequence according to the invention comprises a protein coding portion of the nucleotide sequence shown in SEQ ID NO 1 or a sequence, having an identity of more than 70%, preferably more than 85% and particularly preferred more than 95% of the nucleotide sequence shown SEQ ID NO 1 or a portion thereof having a

length of preferably at least 20 nucleotides, particularly at least 30 nucleotides and most preferably at least 50.

The identity is determined on nucleotide or protein level as follows:

$$I = n : L,$$

wherein

I represents the identity in percent

n represents the number of different nucleotides or amino acids between a test sequence and a basic sequence selected from the nucleotide sequence of SEQ ID NO 1, the amino acid sequence SEQ ID NO 2 or a portion thereof, respectively and

L is the length of the basic sequence to be compared with a test sequence.

A polynucleotide of the present invention may be obtained from mammalian, e.g. human cells or from a cDNA library or a genomic library derived from mammalian, e.g. human cells. In particular, the polynucleotide described herein may be isolated from cDNA libraries (PENCNOTO7, BLADNOTO9, PROSTUTO8, BRSTNOT27, MIXDNOPO1, ESOGNOTO4, PENCNOTO3) available from Incyte Inc. The cDNA insert shown in SEQ ID NO 1 is 3943 base pairs (bp) in lenght and contains an open reading frame encoding a protein 492 amino acids in lenght. The predicted amino acid sequence of the polypeptide of the present invention shares 38% identical amino acids with human heparanase (Figure 1). The 5´-end of the cDNA of the present invention is incomplete; the predicted mature protein is complete as inferred from homology to human heparanase. Electronic expression (Northern) analysis implicates preferential expression of the

polynucleotide of the present invention in nervous system and male genitalia tissues (Figure 2).

The present invention further relates to variants of the herein described polynucleotide which code for fragments, analogs and derivatives of the polypeptide having the deduced amino acid sequence of SEQ ID NO 2. The present invention also relates to polynucleotide probes constructed from the polynucleotide sequence of SEQ ID NO 1 or a segment of SEQ ID NO 1. Variants of the herein described polynucleotide include deletion variants, substitution variants and addition or insertion variants.

The present invention also includes polynucleotides, wherein the coding sequence for the polypeptide, or a segment thereof, may be fused in the same reading frame to a polynucleotide sequence which aids the expression or secretion of a polypeptide from a host cell, or which allows the purification of the polypeptide of the present invention (i.e. a poly-histidin-tag, a hemagglutinin tag, a GST-tag).

A process for the preparation of a polynucleotide according to the present invention represents an aspect of the present invention. Such a process may comprise chemical synthesis, recombinant DNA technology, polymerase chain reaction or a combination of these methods. Preferably the polynucleotide is obtained by means of an amplification reaction, e.g. a PCR using sequence-specific oligonucleotide primers, from a suitable source as described above.

The polypeptide of the present invention may be a recombinant polypeptide, a natural polypeptide or a synthetic polypeptide. The functional fragment, derivative or analog of the present invention may be one in which one or more amino acids are substituted with another amino acid, or one in which one or more of the amino acid residues includes a substituent group, or one in which the polypeptide is fused with another compound (i.e. polyethylene

glycol), or one in which additional amino acids are fused to the polypeptide (i.e. a leader sequence, a secretory sequence, a purification tag).

The present invention also relates to a recombinant vector comprising a polynucleotide of the present invention. Preferably, such a vector is an expression vector, i.e. a vector comprising the polynucleotide of the present invention operatively linked to a suitable expression control sequence. The vector may be a prokaryotic or eukaryotic vector. Examples of prokaryotic vectors are chromosomal vectors such a bacteriophages and extrachromosomal vectors such as plasmids, wherein circular plasmid vectors are particulary preferred. Suitable prokaryotic vectors are disclosed, e.g. in Sambrook et al., supra, Chapters 1-4. On the other hand, the vector may be a eukaryotic vector, e.g. a yeast vector or a vector suitable for expression in higher cells, e.g. insect cells, plant cells or vertebrate cells, particularly mammalian cells. Preferred examples of eukaryotic vectors are plasmids or viral vectors. Suitable eukaryotic vectors are disclosed in Sambrook et al., supra, Chapter 16.

Furthermore, the present invention relates to a cell which contains at least one heterologous copy of a polynucleotide or a vector as defined above. The polynucleotide or the vector may be inserted into the cell by known means, e.g. by transformation (this term also including transfection, electroporation, lipofection, infection etc.). The cell may be a eukaryotic or a prokaryotic cell. Methods for transforming cells with nucleic acids are generally known and need not be explained in detail. Examples for preferred cells are eukaryotic cells, particulary vertebrate and more particulary mamalian cells.

Another aspect of the present invention relates to a recombinant process for the preparation of a polypeptide of the present invention, said process comprising cultivation of a host cell transformed with a polynucleotide or a vector as described above under conditions suitable for performing

expression of the polypeptide, and isolation of the thus-expressed polypeptide from the cell or from the culture supernatant. The host cells can be cultured in conventional nutrient media modified as appropriate for selecting transformants, amplifying the polynucleotide or the vector or purification of the polypeptide.

The thus-expressed polypeptide of the present invention may be recovered and purified from recombinant cell cultures by methods used heretofore, including detergent homogenates, Heparin-Sepharose chromatography, cation exchange chromatography, Con A-Sepharose chromatography, gel-filtration chromatography, Ni-chelating chromatography, glutathion-sepharose (agarose) chromatography, hydrophobic interaction chromatography, and antibody affinity chromatography.

A polypeptide of the present invention may be a purified product naturally expressed from a high expressing cell line, or a product of chemical synthesis, or produced by recombinant techniques from a prokaryotic or eukaryotic host. Depending on the host employed in a recombinant production procedure, a polypeptide of the present invention may be glycosylated or non-glycosylated.

Another aspect of the present invention relates to an oligonucleotide or a derivative thereof, which hybridizes under stringent conditions with the nucleotide sequence set forth in SEQ ID NO 1. Such an oligonucleotide may have a length of, e.g., from about 5, preferably from about 15 to about 100 or even several hundred nucleoside units or analogs thereof, depending on the intended use.

An oligonucleotide of the invention may be used as a cloning primer, or as a PCR primer, or as a sequencing primer, or as a hybridization probe. Another use relates to stimulating or inhibiting expression of a polypeptide of the present invention in vivo by the use of sense or anti-sense

technology. These technology can be used to control gene expression through triple-helix formation on double-stranded DNA or anti-sense mechanisms on RNA, both of which methods are based on binding of such an oligonucleotide to DNA or RNA. Still another use of oligonucleotides, particularly RNA oligonucleotides relates to an expression control by using ribozyme technology. The oligonucleotides can be delivered to cells by procedures in the art either directly or such that the anti-sense or ribozyme RNA or DNA may be expressed in vivo to inhibit production of a polypeptide of the present invention. Anti-sense constructs or ribozymes to a polynucleotide of the present invention inhibit the action of a polypeptide of the present invention and may be used for treating certain disorders, for example, cancer and cancer metastasis.

Further, such oligonucleotides can be used to detect the presence or absence of a polynucleotide of the present invention and the level of expression of such a polynucleotide. Furthermore, such oligonucleotide can be used for the detection of mutations within the gene encoding the polypeptide of the present invention. Mutations within the gene may be correlated with disease or prognosis of disease. Therefore, such oligonucleotides are useful as diagnostic markers for the diagnosis of disorders such as cancer, cancer metastasis, and aberrant angiogenesis.

The polypeptides, their functional fragments, derivatives or analogs thereof, or a cell expressing them, or the polynucleotide or fragments thereof, can be used as an immunogen to produce antibodies thereto. Therefore, the present invention relates to an antibody which specifically recognizes and binds to a polypeptide of the invention.

Such an antibody can be, for example, a polyclonal or a monoclonal antibody. The present invention also includes chimeric, single chain and humanized antibodies, as well as Fab fragments. Various procedures known in the art may be used for the production of such antibodies and fragments.

Polyclonal antibodies may be obtained by immunizing experimental animals with suitable polypeptide or peptide antigens optionally coupled to a carrier and isolating the antibodies from the immunized animals. Monoclonal antibodies may be obtained by the hybridoma technique developed by Köhler and Milstein. Methods for generating polyclonal and monoclonal antibodies, respectively, are generally known and need not be explained in detail (Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988).

Such an antibody can be used for isolating the polypeptide from a tissue expressing that polypeptide. An antibody specific to a polypeptide of the present invention may further be used to inhibit the biological action of the polypeptide by binding to the polypeptide. In this manner, the antibodies may be used in therapy, for example to treat cancer. The cancer therapy may be carried out according to the protocols described by Weiner (Semin. Oncol. 26 (1999), 41-50) or references cited therein.

Further, such antibodies can detect the presence or absence of a polypeptide of the present invention and the level of concentration of such a polypeptide and, therefore, are useful as diagnostic markers for the diagnosis of disorders such as cancer, cancer metastasis, and aberrant angiogenesis.

In a further aspect, the present invention relates to a method for identifying a substance capable of modulating the biological activity or expression of a polypeptide of the present invention. Thus, the present invention is directed to a method for identifying antagonists and inhibitors, as well as agonists and stimulators of the function or activity or expression of a polypeptide of the present invention.

For example, an antagonist may bind to a polypeptide of the present invention and inhibit or eliminate its function. The antagonist, for example,

could be an antibody or an high-affinity oligonucleotide or a peptide against the polypeptide which eliminated the glucuronidase activity of the polypeptide by binding to the polypeptide. An example of an inhibitor is a low molecular weight molecule which inactivates the polypeptide by binding to and occupying the catalytic site, thereby making the catalytic site inaccessible to a substrate, such that the biological activity of the polypeptide is prevented.

Antagonists and inhibitors may be used to treat cancer, cancer metastasis, and aberrant angiogenesis by preventing the polypeptide from functioning to break down heparan sulfate proteoglycan from extracellular matrix.

The antagonists and inhibitors identified by the method as described above or derivatives thereof may be employed in a composition with a pharmaceutical acceptable carrier.

In particular, the present invention relates to an assay for identifying the above-mentioned substances, e.g. low molecular weight inhibitors, which are specific to the polypeptides of the present invention and prevent them from functioning or prevent their expression. Either natural or synthetic carbohydrate substrates would be used to assess endo-glucuronidase activity of the polypeptide.

A further aspect relates to a polynucleotide or a polypeptide according to the present invention for use in medicine. In particular, the invention relates to the use of a polypeptide or a polynucleotide according to the present invention in the preparation of a pharmaceutical composition for the treatment of a disease resulting from shortage or lack of said polypeptide. Instead of or in addition to a polynucleotide or a polypeptide of the present invention, an agonist of the polypeptide or an expression inducer / enhancer of such a polypeptide may be used for the medicinal purposes. Such diseases are, for example, trauma, autoimmune diseases, skin diseases,

cardiovascular diseases, and nervous system diseases. The polynucleotide of the present invention may be used in gene therapy. The gene therapy may be carried out according to protocols described by Beutler (Biol. Blood Marrow Transplant 5 (1999), 273-276) or Gomez-Navarro et al., (Eur. J. Cancer 35 (1999), 867-885) or references cited therein.

Another aspect relates to an antibody according to the present invention or a fragment thereof for use in medicine. In particular, the invention relates to the use of an antibody according to the present invention in the preparation of a pharmaceutical composition for the treatment of a disease resulting from excessive activity or overexpression of a polypeptide of the present invention. Instead of an antibody of the present invention, an antagonist or an inhibitor or an expression inhibitor of such a polypeptide may be used for the medicinal purposes. Such diseases are, for example, cancer, cancer metastasis, angiogenesis and inflammation including arthritis.

Furthermore, the invention is directed to a pharmaceutical composition suitable for administration to a warm-blooded animal inclusive man suffering from a disease resulting from shortage or lack or inactivity of a polypeptide of the present invention, or suffering from a disease resulting from excessive activity or overexpression of a polypeptide of the present invention.

Since the polynucleotide of the present invention is preverentially expressed in male genitalia tissues modulation of expression and/or activity of the encoded polypeptide may be used for medicinal intervention in male genitalia function (i. e. male fertility control, erectile dysfunction).

EXAMPLES

Example 1: Identification of a polynucleotide of the present invention

Using the published sequence of human heparanase (AAD 54941.1) three Incyte templates (i.e. assemblies of Incyte ESTs) could be identified to share significant homology to the human heparanase. Some of these ESTs of each template were ordered from Incyte. Determination of the nucleotide sequence of the 3´- and 5´-ends of each EST clone revealed more novel sequence information which lead to further two assemblies from Incyte clones. Combining this sequence information and sequence information from own sequencing efforts of these Incyte clones enabled us to assemble a novel paralogue, human heparanase-related polypeptide, of human heparanase. The novel sequence comprises 3943 bp and the identified coding sequence ranges from 1 bp - 1479 bp (including STOP codon). The 5´ end is still open as both coding region analysis (as detemined by the program ESTSCAN) and homology to human heparanase suggest.

Example 2: Electronic expression analysis

Based on the number of ESTs for a given tissue one can estimate or predict a measure for the in vivo expression level of the given gene in this given tissue.

"Electronic-northern" is a bioinformatic method that firstly identifies the overall number for all ESTs for a given tissue (so-called "pool-size") that are in the database and secondly the number of ESTs from that tissue which correspond only to the query sequence.

This is done by a BLAST (NCBI BLAST v. 2.0.10; Altschul et al., Nucleic Acid Res. (1997) 25, 3389-3402) search using the cDNA of the gene of interest as query and the human EST database (LifeSeqGold from Incyte) as

data source. The search parameters were E = 1e-30. A SQL-query in the database retrieves then for each EST coming up from the search its tissue source and the pool-size for each tissue.

This data is believed to correlate with the expression level in vivo. Statistical analysis (normalisation on pool-size and confidence interval determination) helps here to estimate the reliability of the data and to compare the expression level between different tissues. The reliability of this prediction method increases usually with the number of hits/tissue and the pool-size of a tissue.

Example 3: Expression of the polynucleotide

The coding region of the polynucleotide given in SEQ ID NO 1 was amplified by PCR using 5'-primer HepR1 (5'-GAC AGG AGA CCC TTG CCT GTA GAC-3') and 3'-primer HepR2 (5'-ATA GTC GAG TTA TCG GTA GCG GCA GGC CAA AGC-3') and DNA isolated from clones #3207535H1 and #3385824H1 the database LifeSeqGold from Incyte Inc. issue of Oct/Nov 1999 as template DNA. The 1488 bp DNA was phosphorylated using T4 polynucleotide kinase followed by restriction digestion using XhoI. The fragment was ligated in frame into pISP-myc vector providing an N-terminal immune globuline signal sequence followed by an myc-tag epitope. Upon restriction digestion using HindIII and XhoI the fragment was ligated into the appropriate sites of expression vector pCEP4 (Invitrogen) generating expression vector HepR-pCEP. HepR-pCEP was stably transfected into MCF7, MBA-231, and MBA-468 breast carcinoma cell lines, as well as in CHO cells. The recombinant protein was detected using an anti-myc-tag epitope antibody.

For expression in the insect cells, the PCR-fragment was released from pISP-myc vector using EcoRI and XbaI. The fragment was cloned into

pVL1392 baculovirus transfer vector generating HepR-pVL vector and transfected into Sf9 insect cells.

Example 4: Production of antibodies

Polypeptide purified from infected Sf9 insect cells using expression vector HepR-pVL of example 3 was used for immunization of mice and rabbits, respectively, using standard procedures (Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988).

Claims

1. A polynucleotide comprising
   (a) the sequence as set forth in SEQ ID NO 1 or at least the protein coding portion thereof,
   (b) a nucleotide sequence corresponding to the sequence of (a) in the scope of the degeneracy of the genetic code, or
   (c) a nucleotide sequence hybridizing under stringent conditions with a sequence from (a) and/or (b).

2. The polynucleotide of claim 1 encoding a polypeptide having the biological activity of an endo-glucuronidase.

3. The polynucleotide of claim 1 or 2 having an identity of at least 70% to the nucleotide sequence as set forth in SEQ ID NO 1 or a fragment thereof.

4. The polynucleotide of any one of claims 1 to 3 which is a DNA, an RNA or a nucleic acid analog.

5. A recombinant vector comprising at least one copy of the polynucleotide of any one of claims 1-4.

6. The vector of claim 5 which is an expression vector.

7. A cell which is transformed with the polynucleotide of any one of claims 1-4 or with the vector of any one of claims 5-6.

8. A polypeptide which is encoded by the polynucleotide of any one of claims 1-4.

9. The polypeptide of claim 8 comprising

(a) the amino acid sequence as set forth in SEQ ID NO 2, or

(b) an amino acid sequence having a identity of at least 70% to the amino acid sequence of (a) or a fragment thereof.

10. The polypeptide of claim 8 or 9 having an endo-glucuronidase activity.

11. The polypeptide of any one of claims 8-10 or a fragment thereof being capable of eliciting specific antibodies.

12. A process for the preparation of a polypeptide according to any one of claims 8-11, said process comprising chemical synthesis, recombinant DNA technology or a combination of these methods.

13. A process for the preparation of a polynucleotide according to any one of claims 1-3, said process comprising chemical synthesis, recombinant DNA technology, polymerase chaim reaction or a combination of these methods.

14. An antibody or a oligopeptide or a oligonucleotide or derivatives thereof which specifically recognizes and binds to a polypeptide as defined in claims 8-11.

15. A polynucleotide of any one of claims 1-4 or a polypeptide of any one of claims 8-11 for use in medicine.

16. Use of a polynucleotide of any one of claims 1-4 or a polypeptide of any one of claims 8-11 in the preparation of a pharmaceutical composition for the treatment of a disease resulting from shortage or lack or inactivity of said polypeptide.

17. A method of treatment of a disease resulting from shortage or lack, or inactivity of a polypeptide as defined in claims 8-11, said method comprising administration of a suitable amount of a polynucleotide of any one of claims 1-4 or a polypeptide of any one of claims 8-11.

18. A method of treatment of a disease resulting from excessive activity or overexpression of a polypeptide as defined in claims 8-11, said method comprising administration of a suitable amount of an antibody or a oligopeptide or a oligonucleotide or derivatives thereof as defined in claim 14.

19. A method for identifying a substance capable of modulating the biological activity or expression of a polypeptide as defined in claims 8-11 in a cell, said method comprising contacting the polypeptide or a functional derivative, a functional fragment or a functional analog thereof, or a cell capable of expressing the polypeptide, with at least one compound or agent whose ability to modulate the biological activity or expression of said polypeptide, functional derivative, functional fragment or functional analog is sought to be investigated, and determining the change of the biological activity or the expression of said polypeptide, derivative or fragment caused by the substance.

20. The method of claim 19, further comprising formulating a pharmaceutical composition comprising as an active agent a substance which has been identified as a modulator or a derivative thereof.

21. An assay system for testing a substance for its capability of binding to or having functional effects on a polypeptide as defined in claims 8-11, said assay system comprising the polypeptide, or a functional derivative, a functional fragment or a functional analog thereof, or a cell capable of expressing the polypeptide or a functional derivative, a

functional fragment or a functional analog and optionally means for determining a response caused by the substance.

22. A substance obtainable by a method as defined in claim 19 or 20, said substance being an agonist or antagonist of a polypeptide as defined in claims 8-11.

23. Use of a polynucleotide of any one of claims 1-4 or a fragment or derivative thereof for modulating the expression of a polypeptide as defined in claims 8-11 in a cell.

24. Use of a polynucleotide of any one of claims 1-4 in gene therapy.

25. Use of an antibody or a oligopeptide or a oligonucleotide or a derivative thereof as defined in claim 14 or of a polynucleotide or a fragment or derivative thereof of any one of claims 1-4 for diagnosis of a disease resulting from shortage or overexpression of a polypeptide a defined in claims 8-11.

Figure 1. Alignment of amino acid sequences of the polypeptide of the present invention with that of human heparanase.

An alignment

between human heparanase AAD54941.1 (543 AA)
and HUMAN_HEPARANASE_LIKE171199 (492 AA) is shown below and reveals nearly 38% identity in 529 AA overlap.
From the alignment it can be inferred that only the signal peptide is missing. The bold letters in the human heparanase sequence (the upper one) indicates the predicted signal peptide
(Most likely signal peptide cleavage site of human heparanase is between pos. 35 and 36: AQA-QD)

Smith-Waterman score: 1155; 38.563% identity in 529 aa overlap

aad54941.1 = published human heparanase
novel.con1_orf1 = novel heparanase-related

```
aad54941.1
MLLRSKPALPPPLMLLLLGPLGPLSPGALPRPA..QAQDVVDLDFFTQEPLHLVSPSFLS
novel.con1_orf1
~~~~~~~~~~~~~~~~~~~~~~DRRPLPVDRAAGLKEKTLILLDVSTKNPVRTVNENFLS

aad54941.1
VTIDANLATDPRFLILLGSPKLRTLARGLSPAYLRFGGTKTDFLIFDPKKESTFEERSYW
novel.con1_orf1
LQLDPSIIHD.GWLDFLSSKRLVTLARGLSPAFLRFGGKRTDFLQF.............

aad54941.1
QSQVNQDICKYGSIPPDVEEKLRLEWPYQEQLLLREHYQKKFKNSTYSRSSVDVLYTFAN
novel.con1_orf1
.QNLRNPAKSRGGPGPDYYLK.....NYEDDIVRSDVALDKQKGCKIAQHPDVML.....

aad54941.1
CSGLDLIFGLNALLRTADLQWNSSNAQLLLDYCSSKGYNISWELGNEPNSFLKKADIFIN
novel.con1_orf1
.................ELQREKAAQMHLVLLKEQFSNTYSNLILTEPNNYRTMHGRAVN

aad54941.1
GSQLGEDFIQLHKLLRK.STFKNAKLYGPDVGQPRRKTAKMLKSFLKAGGEVIDSVTWHH
```

```
novel.c n1_orf1
GSQLGKDYIQLKSLLQPIRIYSRASLYGPNIGRPRKNVIALLDGFMKVAGSTVDAVTWQH

aad54941.1
YYLNGRTATREDFLNPDVLDIFISSVQKVFQVVESTRPGKKVWLGETSSAYGGGAPLLSD
novel.con1_orf1
CYIDGRVVKVMDFLKTRLLDTLSDQIRKIQKVVNTYTPGKKIWLEGVVTTSAGGTNNLSD

aad54941.1
TFAAGFMWLDKLGLSARMGIEVVMRQVFFGAGNYHLVDENFDPLPDYWLSLLFKKLVGTK
novel.con1_orf1
SYAAGFLWLNTLGMLANQGIDVVIRHSFFDHGYNHLVDQNFNPLPDYWLSLLYKRLIGPK

aad54941.1
VLMASVQGSKRR.........KLRVYLHCTNTDNPRYKEGDLTLYAINLHNVTKYLRLPY
novel.con1_orf1
VLAVHVAGLQRKPRPGRVIRDKLRIYAHCTNHHNHNYVRGSITLFIINLHRXRKKIKLAG

aad54941.1
PFSNKQVDKYLLRPLGPHGLLSKSVQLNGLTLKMVDDQTLPPLMEKPLRPGSSLGLPAFS
novel.con1_orf1
TLRDKLVHQYLLQPYGQEGLKSKSVQLNGQPLVMVDDGTLPELKPRPLRAGRTLVIPPVT

aad54941.1      YSFFVIRNAKVAACI~~
novel.con1_orf1 MGFYVVKNVNALACRYR
```

Figure 2. Results from electronic expression analysis

	human heparanase-related polynucleotide 38 hit sequences mapped to 26 clones
Tissue category specific hits	
Cardiovascular System:	3 / 229661
Connective Tissue:	0 / 112794
Digestive System:	4 / 349101
Embryonic Structures:	2 / 84199
Endocrine System:	0 / 179602
Exocrine Glands:	1 / 236109
Genitalia, Female:	4 / 299477
Genitalia, Male:	**10 / 380251**
Germ Cells:	0 / 15257
Hemic and Immune System:	2 / 604773
Liver:	0 / 78335
Musculoskeletal System:	0 / 131798
Nervous System:	**7 / 612689**
Pancreas:	0 / 85915
Respiratory System:	0 / 312810
Sense Organs:	0 / 19264
Skin:	0 / 60395

Stomatognathic System:	0 / 10997
Unclassified/Mixed:	1 / 63078
Urinary Tract:	4 / 212571

Organ specific clone hits	
Adrenal Glands:	0 / 62919
Bladder:	3 / 58862
Blood:	2 / 282429
Blood Vessels:	3 / 130510
Bone Marrow:	0 / 45994
Bones:	0 / 41311
Brain:	3 / 479254
Breast:	1 / 236109
Bronchi:	0 / 32669
Cartilage:	0 / 21947
Connective Tissue:	0 / 133893
Ear:	0 / 3156
Embryo:	0 / 2993
Esophagus:	2 / 15236
Eye:	0 / 13632
Fallopian Tubes:	0 / 4432
Fetus:	2 / 24184
Gallbladder:	0 / 21391

Germ Cells:	0 / 15257
Heart:	0 / 99151
Intestine:	0 / 3164
Intestine, Large:	1 / 193491
Intestine, Small:	0 / 86067
Kidney:	1 / 149891
Larynx:	0 / 5513
Liver:	0 / 88010
Lung:	0 / 267484
Lymphoid Tissue:	0 / 35954
Mixed Tissues:	1 / 145333
Muscles:	0 / 39730
Nervous Tissue:	0 / 98790
Nose:	0 / 8612
Ovary:	0 / 115176
Pancreas:	0 / 102662
Parathyroid Glands:	0 / 25033
Penis:	**7 / 40938**
Pineal Body:	0 / 3607
Pituitary Gland:	0 / 12271
Placenta:	0 / 57022
Prostate:	3 / 233994
Salivary Glands:	0 / 3951

Seminal Vesciles:	0 / 14055
Skin:	0 / 60395
Spinal Cord:	4 / 23932
Spleen:	0 / 54556
Stomach:	1 / 29752
Synovial Membranes:	0 / 36053
Testis:	0 / 90864
Thymus Gland:	0 / 50982
Thyroid Gland:	0 / 45573
Tongue:	0 / 3351
Tonsil / Adenoids:	0 / 55437
Ureter:	0 / 3818
Uterus	4 / 167760

SEQUENCE LISTING

SEQUENCE ID NO 1:

Nucleotide sequence listing of cDNA encoding human Heparanase-like polypeptide

Length:	3943 bp
Coding sequence region:	1-1476 bp
STOP codon:	1477-1479 bp

Two putative polyadenylation sites are indicated by underlined letters.

```
   1  gacaggagac ccttgcctgt agacagagct gcaggtttga aggaaaagac
  51  cctgattcta cttgatgtga gcaccaagaa cccagtcagg acagtcaatg
 101  agaacttcct ctctctgcag ctggatccgt ccatcattca tgatggctgg
 151  ctcgatttcc taagctccaa gcgcttggtg accctggccc ggggactttc
 201  gcccgccttt ctgcgcttcg ggggcaaaag gaccgacttc ctgcagttcc
 251  agaacctgag gaacccggcg aaaagccgcg ggggcccggg cccggattac
 301  tatctcaaaa actatgagga tgacattgtt cgaagtgatg ttgccttaga
 351  taaacagaaa ggctgcaaga ttgcccagca ccctgatgtt atgctggagc
 401  tccaaaggga gaaggcagct cagatgcatc tggttcttct aaaggagcaa
 451  ttctccaata cttacagtaa tctcatatta acagagccaa ataactatcg
 501  gaccatgcat ggccgggcag taaatggcag ccagttggga aaggattaca
 551  tccagctgaa gagcctgttg cagcccatcc ggatttattc cagagccagc
 601  ttatatggcc ctaatattgg gcggccgagg aagaatgtca tcgccctcct
 651  agatggattc atgaaggtgg caggaagtac agtagatgca gttacctggc
 701  aacattgcta cattgatggc cgggtggtca aggtgatgga cttcctgaaa
 751  actcgcctgt tagacacact ctctgaccag attaggaaaa ttcagaaagt
 801  ggttaataca tacactccag gaaagaagat ttggcttgaa ggtgtggtga
 851  ccacctcagc tggaggcaca aacaatctat ccgattccta tgctgcagga
 901  ttcttatggt tgaacacttt aggaatgctg gccaatcagg gcattgatgt
 951  cgtgatacgg cactcatttt ttgaccatgg atacaatcac ctcgtggacc
1001  agaattttaa cccattacca gactactggc tctctctcct ctacaagcgc
1051  ctgatcggcc ccaaagtctt ggctgtgcat gtggctgggc tccagcggaa
1101  accacggcct ggccgagtga tccgggacaa actaaggatt tatgctcact
1151  gcacaaacca ccacaaccac aactacgttc gagggtccat tacacttttt
1201  atcatcaact tgcatcgakc aagaaagaaa atcaagctgg ctgggactct
1251  cagagacaag ctggttcacc agtacctgct gcagccctat gggcaggagg
1301  gcctaaagtc caagtcagtg caactgaatg gccagccctt agtgatggtg
1351  gacgacggga ccctcccaga attgaagccc cgcccccttc gggccggccg
1401  gacattggtc atccctccag tcaccatggg cttttatgtg gtcaagaatg
```

1451 tcaatgcttt ggcctgccgc taccgaTAAg ctatcctcac actcacggct
1501 accagtgggc ctgctgggct gcttccactc ctccactcca gtagtatcct
1551 ctgttttcag acatcctagc aaccagcccc tgctgcccca tcctgctgga
1601 atcaacacag acttgctctc caaagagact aaatgtcata gcgtgatctt
1651 agcctaggta ggccacatcc atcccaaagg aaaatgtaga catcacctgt
1701 acctatataa ggataaaggc atgtgtatag agcagaatgt ttcccttcat
1751 gtgcactatg aaaacgagct gacagcacac tcccaggaga aatgtttcca
1801 gacaactccc catgatcctg tcacacagca ttataaccac aaatccaaac
1851 cttagcctgc tgctgctgct gccctcagag gaagatgagg aaggaaaaaa
1901 actgggtgga cctacaaaaa cccatcctct cccaactcct tcttctctgc
1951 ctctttcttg ctgctgccct gagtttttg acacatctct ttccataggg
2001 gagtaatggg tgtgtcagcc ctggcctgct gggagagctg tttgtatgat
2051 ttcccggctg atgtatgagc gtgcgcatct gggttcctga cagtggcatc
2101 catcactggc agttcttctg ggaagcgggt gcttcaaaag taaaattaca
2151 atcacactcc agatttggta agaaggttct attcctctgt gaatccagat
2201 tcccccagag ttgtaatggg agtcaagtaa caatattcat tgagtggaga
2251 gcagtttatt aggcacaaca aaaagtaatc atcattcttc atgttgctat
2301 gagggagagt ttgagtacaa agagaaagca tactgaaaca tcaggtacac
2351 acacacaccc caactggaca aagcaaatta gacctctcca aaattaagag
2401 aatattaggg gctctatagg gtaagccttt aattgtttgg ttaactcaaa
2451 tcattatttt taaaaaagaa gaaaaaagtg tgaatcaagg tcatcactgg
2501 aagacacaac tgaatctaac ctttttgcct cttcccaagt agcctatttg
2551 agctagaaca aaactttgtt agccattttg ggagagaata gggaatctag
2601 agaatgaaga tctgcccaaa actatggaat ggtaggtagg aagcttctga
2651 gttgggcagg tgtgaagtgg gggatgagga cgttctatat gattcaaggg
2701 gcatgagggt ctttgccaat gagctacagc tgaaatgact ttcttttctg
2751 gggatgtgat tttctttctc aggataaatg acaggaatga tgcttttgtt
2801 agaaggagga gagatttgac actgttccaa gtgagacagt gatacaattt
2851 ctgctgtttg tgaaaggaca ggaatggggy gggggcaagg cagggttgcc
2901 tagggcagag actagggagg ctgcctaaga cgcacacgga gttaaggatt
2951 tgggccaagt ctgcaaagtg agagatggaa gggagattag accaaagagg
3001 agggagagaa ttctgagctt ggagaacggt ggatttggga gagggaagct
3051 gactacctaa ttccaggaag cgaggggacc gggttttgac atgcttatca
3101 ttaagcacag gaggaacagc atacagcaga tgtactacag cgagcaagaa
3151 agggagagcc cgaggaccag gctgcaccag gtcagtggct gtgctcagca
3201 tggaagcaac tggagagaga ggggcagacc ctgagacygc cctgcaaggc
3251 tgcccagaag ggacccgttt ctctgggacc aggcacctcc cactgaggct
3301 tcagctctga gagggcagga aagtgaagta ccaagatggg ggcggggcgg
3351 ggggtaggaa ataagagaaa gaagaaacag attgacaggc caaagtgagg
3401 aaaagagagg aaaagagaaa tgagactaaa aggtcgttcc cccaactgtt
3451 aaaaatgtgt gcagatatca acgtctcttc tacatactgg tacaggtgcg
3501 actgcagggc cccctgatat aacaagagta accaaaggtc cctaagagcc
3551 tggccctggg gacctatggt ttgctttgcg tccttagtaa ccccatgata
3601 aaggggtact actgttatcc ccatttttcc tacgaggcat ggagaggatc
3651 catggctcgc cccaggggca cccggggaaa tgggttgccg agcgcgaaat

```
3701  aatccagagc ctgcccactc agccacaagg ctcagcggct ccacaggtcc
3751  agacacctcc ttcacatctt tgtaggttct gctcattcag aacagccaga
3801  actccactca aacacacttt ctgtaaataa gtgttgattt ttttttacta
3851  aaccttgcag aatatgggta attcctgctt cttttatctt tctctgtgta
3901  ttaaatgctg ctctcacgag atttaagttt tgtttatttt tta
```

SEQUENCE ID NO 2:

Amino acid sequence listing of human Heparanase-related polypeptide

Translation product

Length: 492

```
  1  DRRPLPVDRA AGLKEKTLIL LDVSTKNPVR TVNENFLSLQ LDPSIIHDGW
 51  LDFLSSKRLV TLARGLSPAF LRFGGKRTDF LQFQNLRNPA KSRGGPGPDY
101  YLKNYEDDIV RSDVALDKQK GCKIAQHPDV MLELQREKAA QMHLVLLKEQ
151  FSNTYSNLIL TEPNNYRTMH GRAVNGSQLG KDYIQLKSLL QPIRIYSRAS
201  LYGPNIGRPR KNVIALLDGF MKVAGSTVDA VTWQHCYIDG RVVKVMDFLK
251  TRLLDTLSDQ IRKIQKVVNT YTPGKKIWLE GVVTTSAGGT NNLSDSYAAG
301  FLWLNTLGML ANQGIDVVIR HSFFDHGYNH LVDQNFNPLP DYWLSLLYKR
351  LIGPKVLAVH VAGLQRKPRP GRVIRDKLRI YAHCTNHHNH NYVRGSITLF
401  IINLHRXRKK IKLAGTLRDK LVHQYLLQPY GQEGLKSKSV QLNGQPLVMV
451  DDGTLPELKP RPLRAGRTLV IPPVTMGFYV VKNVNALACR YR
```

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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PCT

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(21) International Application Number: PCT/EP00/12909

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18 December 2000 (18.12.2000)

(25) Filing Language: English

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99125831.0 23 December 1999 (23.12.1999) EP

(71) Applicant *(for all designated States except US)*: **SCHERING AKTIENGESELLSCHAFT** [DE/DE]; Müllerstrasse 178, 13353 Berlin (DE).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **SIEMEISTER, Gerhard** [DE/DE]; Reimerswalder Steig 26, 13503 Berlin (DE). **WEISS, Bertram** [DE/DE]; Im Schwarzen Grund 4, 14195 Berlin (DE).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report:
14 February 2002

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/48161 A3

(54) Title: HUMAN HEPARANASE-RELATED POLYPEPTIDE AND NUCLEIC ACID

(57) Abstract: The present invention relates to newly identified polynucleotides, and polypeptides encoded by such polynucleotides, the use of such polypeptides, as well as the production of such polynucleotides and polypeptides. More particularly, a polypeptide of the present invention is a heparanase-related endoglucuronidase. The invention also relates to vectors and host cells comprising a polynucleotide of the invention. Furthermore, the invention relates to antibodies directed to polypeptides according to the present invention and to pharmaceutical compositions and diagnostic reagents comprising such antibodies, polypeptides or polynucleotides. The invention further relates to a method of altering, modifying or otherwise modulating the level of expression of the heparanase-related endoglucuronidase in a cell or in a organism. A further aspect of the invention are assay systems suitable for identifying modulators, e.g. agonists or antagonists of such polypeptides.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/12909

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N9/24

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, EMBL, WPI Data, PAJ, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	STRAUSBERG R: "qg97h02.x1 Soares_NFL_T_GBC_S1 Homo sapiens cDNA clone IMAGE:1843155 3', mRNA sequence" EMBL DATABASE, 28 October 1998 (1998-10-28), XP002155088 the whole document ---	1,3-7
X	DATABASE EMBL 'Online! Accession number AI377012, 28 January 1999 (1999-01-28) STRAUSBERG R.: "tc28e02.x1 Soares_total_fetus_Nb2HF8_9w Homo sapiens cDNA clone IMAGE:2065946 3', mRNA sequence" XP002170438 the whole document ---	1,3-7

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 July 2001	17/07/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Armandola, E

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category ˘	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	DATABASE EMBL 'Online! Accession Number AF282886, 16 October 2000 (2000-10-16) MCKENZIE ET. AL.: "Homo sapiens heparanase-like protein HPA2b mRNA, complete cds" XP002170439 the whole document	1-11
P,X	MCKENZIE E ET AL: "Cloning and expression profiling of hpa2, a novel mammalian heparanase family member" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS,ACADEMIC PRESS INC. ORLANDO, FL,US, vol. 276, no. 3, 5 October 2000 (2000-10-05), pages 1170-1177, XP002155087 ISSN: 0006-291X the whole document	1-11
E	WO 01 21814 A (MERCK PATENT GMBH ;DUECKER KLAUS (DE); SIRRENBERG CHRISTIAN (DE)) 29 March 2001 (2001-03-29) SEQ. ID. NO: 1 99.7% identity between nt. 485-1480 the whole document	1-15, 19-21
E	WO 01 00643 A (ITZHAKI HANAN ;MICHAL ISRAEL (IL); PECKER IRIS (IL); INSIGHT STRAT) 4 January 2001 (2001-01-04) SEQ. ID. NO: 5 displays 89.9% identity the whole document	1-14,23
Y	WO 99 21975 A (HAMDORF BRENTON JAMES ;UNIV AUSTRALIAN (AU); FREEMAN CRAIG GEOFFRE) 6 May 1999 (1999-05-06) the whole document	1-19,21, 23-25
Y	WO 99 57153 A (HADASIT MED RES SERVICE ;FRIEDMAN YAEL (IL); PECKER IRIS (IL); FRI) 11 November 1999 (1999-11-11) the whole document	1-19,21, 23-25

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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 20, 22

Present claims 20 and 22 relate to an extremely large number of possible compounds. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the compounds, namely antibodies specific for human heparanase.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 00/12909

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0121814 A	29-03-2001	NONE	
WO 0100643 A	04-01-2001	AU 5244800 A	31-01-2001
WO 9921975 A	06-05-1999	AU 1010999 A	17-05-1999
		BR 9813296 A	22-08-2000
		CN 1280613 T	17-01-2001
		EP 1032656 A	06-09-2000
		US 6242238 B	05-06-2001
		ZA 9809824 A	24-06-1999
WO 9957153 A	11-11-1999	US 6177545 B	23-01-2001
		AU 3870699 A	23-11-1999
		EP 1073682 A	07-02-2001
		NO 996229 A	24-02-2000